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ON THE INFLUENCE OF LOW-CONCENTRATION NITROGEN DIOXIDE ON LIVING ORGANISMS

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[ON THE INFLUENCE OF LOW-CONCENTRATION NITROGEN DIOXIDE ON LIVING ORGANISMS]

Yokohama City Public Nuisance Control Center

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PREFACE

Attention has been focused on the harmfulness of the carbon monoxide contained in automobile exhaust gas, emissions standards and environmental standards have been determined, and gradual progress has been made in cleaning up the pollution caused by carbon monoxide. Most recently, attention has been attracted to pollution consisting chiefly of lead compounds, nitrogen dioxide, hydrocarbons, and ozone. In spite of this, little research is in progress concerning the influences on living organisms.

The results of a study entitled "Experimental Research Concerning the Influence of Automobile Exhaust Gases on Living Organisms", commissioned to the Hygiene Department, Yokohama Municipal University Medical School, have already been published by the Yokohama City Public Nuisance Control Center as its Kōgai shiryō (Environmental Pollution Materials) No. 10 and No. 14. The research results entitled "On the Influence of Low-Concentration Nitrogen Dioxide on Living Organisms" for fiscal year 1969 have been submitted and are herein reported.

March, 1970

Yokohama City Public Nuisance Control Center

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ON THE INFLUENCE OF LOW-CONCENTRATION NITROGEN DIOXIDE ON LIVING ORGANISMS

Yokohama City Public Nuisance Control Center

INTRODUCTION

Problems connected with the occurrence of environmental pollution accompanying the high growth rate of our country's industrial economy and the phenomena of pronounced overcongestion in the cities have become topics of interest in many areas. In particular, much attention is being paid to questions of atmospheric pollution in connection with its social influences over broad areas. As for the adverse influences on the human organism, even though they had been frequently pointed out previously, it appears as if we have just reached the stage where research has finally begun concerning the effects on living organisms. It is particularly difficult to determine the direct causal relationship between low-concentration environmental pollutants and vital reactions, and this is one of the reasons why medical research into atmospheric pollution has lagged behind. It may also be one of the reasons behind the delay in formulating pollution countermeasures in the interests of the human being.

In the past, there have been attempts to take up automobile exhaust gases, which are localized sources of atmospheric pollution in the cities, and to make a few studies concerning their influences on living organisms. Recently, however, the environmental pollution caused by automobile exhaust gases has increased to an unpardonable extent. Even though restrictions on automobile exhaust gases were included among the provisions of the Air Pollutions Control Law, promulgated a few years ago, the situation has not improved to the point where appreciable effects could be anticipated. Subsequently, there have been further tremendous increases in the number of

automobiles, and it is felt that it is now necessary to formulate other, more advanced countermeasures. It is not difficult to imagine, against such a background, that environmental pollution in Japanese cities will tend to move more and more in the direction of a type of pollution in which oxidants such as nitrogen oxides and ozone produced by automobile exhaust gases will be among the chief components. It is entirely possible that, even though there are some differences in the geographical and meteorological conditions, the same type of environmental pollution will occur here as the atmospheric pollution which has occurred in the state of California in the United States.

Nitrogen oxides such as nitrogen dioxide, together with ozone, are edematous gases with powerful irritation of the respiratory tract. Their acute toxic properties have been frequently reported in labor sanitation. However, as was mentioned above, it has only been relatively recently that facts have begun to be discovered concerning the effects of nitrogen dioxide on living organisms in the form of atmospheric pollution. Research reports concerning these matters in the United States have included research concerning the changes in the bronchial and lung tissue pictures and the increased mortality caused by chronic experimental exposure to low-concentration nitrogen dioxide. There have also been research results indicating an increased sensitivity to pathogenic bacteria introduced through the airways in experimental animals exposed over prolonged periods to nitrogen dioxide at extremely low concentrations. In Japan as well, tests of histopathological changes in the lungs have been made, and there have been a few reports concerning the influence on the respiratory functions. However, in some of these studies the concentrations of exposure have been extremely high, producing results which may not be suitable for discovering the effects which would occur at the atmospheric pollution level. We are only at a stage where on part of the influence on nitrogen oxides on living organisms has been clearly established.

We have for some time been engaged in studies aimed at discovering medical indices which would be effective in evaluating the harmfulness of automobile exhaust gases and could be used in formulating countermeasures. Here we report some studies which involved continuously exposing animals to

extremely low concentrations of nitrogen dioxide for the purpose of finding out its influence on their growth and physical strength. We also made some studies of the influence on the blood properties and of the irritation of the lung tissues.

EXPERIMENTAL METHODS

Studies were made with a gas exposure chamber for animal experiments (see the photograph in Yokohama City Public Nuisance Control Center, Kōgai shiryō [Environmental Pollution Materials] No. 10). The animals were repeatedly exposed to low-concentration nitrogen dioxide gas under the conditions described below.

Guinea pigs, rats, and mice were chosen as the experimental animals depending upon the purpose for which they were used. The nitrogen dioxide gas was produced in the laboratory from pieces of metallic copper and sulfuric acid. It was diluted in large quantities of fresh air and was further scrubbed by being passed through a potassium permanganate solution. This was introduced into the gas exposure chamber, and the concentrations were measured with the passage of time by means of the Kitagawa detection tube method and the colorimetric method (Saltzman method). Measurements were made, both to adjust the concentration to a concentration corresponding to the purpose for which the experiment was intended, and also to obtain uniformity of concentration.

Exposure to nitrogen dioxide was performed for three hours a day. Continuous exposure experiments for a period of one month at 5 ppm and for three months at 1 ppm were performed using guinea pigs. At definite intervals during each of these periods, one group of five animals each was killed, and studies were made of their growth process, there hematological properties, the amounts of free histamine in their lungs and blood, and the changes in their lung weights.

Similar experiments in repeated exposures to 0.5 ppm for 40 days were conducted with rats, and studies were made of the influence on their growth

and of the connections between the histamine in the lung tissues and lung disturbances. Studies were also made with mice concerning the amount of moisture in the lungs and the durability of physical strength.

The fluorocolorimetrical method of Shore et al. using o-phthal-aldehyde was used to measure the histamine. The moisture contents in the lungs were sought from the amounts of moisture loss caused by drying for 24 hours at 110° C. The physical strength of the mice was measured by observing them swimming in a bath of water of 25° C. The blood tests were made according to the commonly used methods.

EXPERIMENTAL RESULTS

1. Influence on Growth and Physical Strength

As the experiments progressed, measurements of the bodily weights were taken at definite time intervals in order to determine the growth of the group exposed for three months to 1 ppm of NO_2, the group exposed for one month to 5 ppm, and the group exposed for 40 days to 0.5 ppm. The mean values and rates of increase are shown in Figures 1 and 2.

In the group of rats exposed to 0.5 ppm there seems to be a certain tendency towards growth inhibition during the latter half-period as compared with the control group. However, no stochastically significant results were seen. No differences from the control group were seen in the guinea pigs. It would appear that, during an observation period as short as this, no pronounced disturbances resulting in a reduction of the body weight will appear, partly perhaps because of the low concentrations of exposure in this instance.

In Table 1 and Figure 3 are also shown the results obtained with similar tests with mice who were exposed to 1 ppm for three months. Studies were made of the influence on the durability of physical strength with the progress of the exposure. The durability of physical strength was indicated in terms

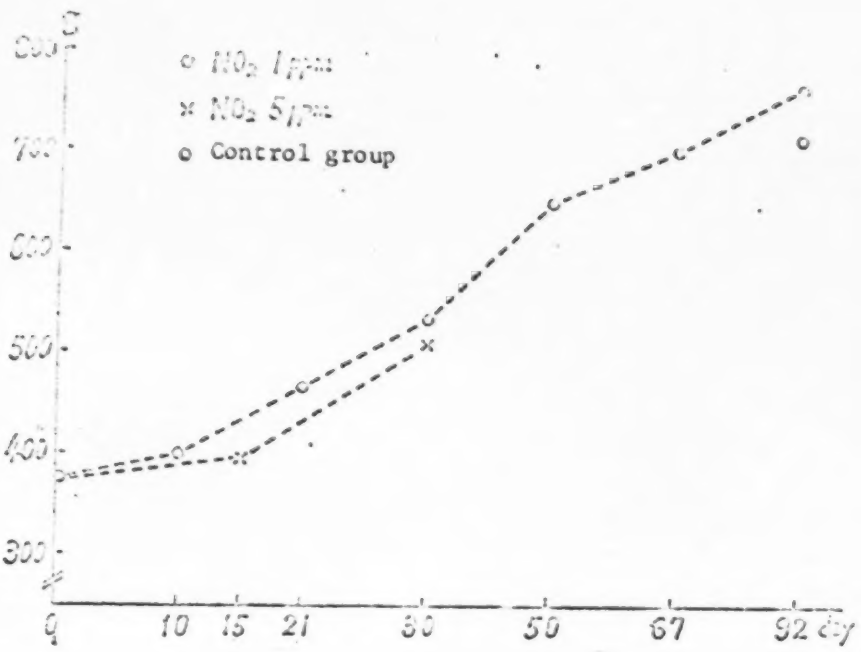

Figure 1. Changes in mean body weights of guinea pigs exposed to NO_2

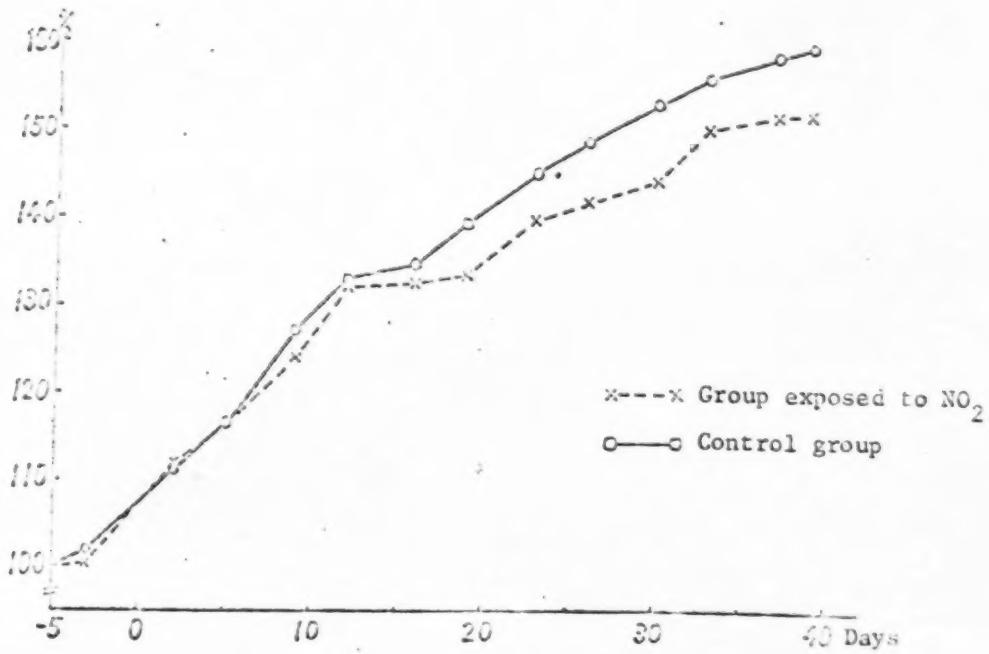

Figure 2. Rate of increase of body weights of rats (NO_2 0.5 ppm)

TABLE 1. TIME OF RETENTION OF PHYSICAL STRENGTH BY MICE (NO_2 1 ppm)
(Swimming method, 25° C)

	Control group	16th day	31st day	47th day	61st day	82nd day
Durability of physical strength (minutes)	420	510	423	286	334	24
	259	420	270	154	337	16
	384	400	529	300	282	14
	449	420	277	690	542	8
		430	305	303	14	15
						11
						12
Mean (M±SD)	378±83	456±42	361±112	347±201	302±189	14±5

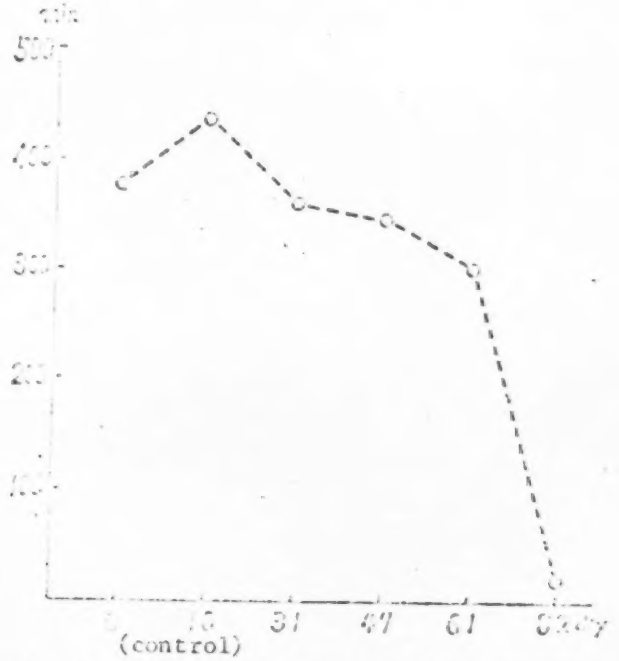

Figure 3. Changes in time of retention of physical strength after exposure to NO_2 (mice, NO_2 1 ppm, swimming method)

of the time until death while swimming in a water bath of 25° C.

As a result, it was found that, during the initial period of exposure, the mice retained the same degree of physical strength as the control group. However, there was a gradual decline as the number of days of exposure increased, and an abrupt decrease in physical strength was observed in a period of about three months.

2. Influence on Blood Properties

Taking into account the affinity of nitrogen dioxide for the erythrocyte system, the studies concerning the influence on the blood properties were chiefly concerning the whole blood specific gravity, the erythrocyte count, the hematocrit value, and the hemoglobin count.

In Figures 4 and 5 are shown the results after 15 days and 30 days of guinea pigs exposed to 5 ppm for one month. (The points in the figures show the results for individuals, and the horizontal lines show the mean value for each group. The identical methods of indication are followed below.) The results for the group exposed to 1 ppm for three months are shown in Figures 6 - 9. Rats were used. The results after 18 and 39 days for the group exposed to 0.5 ppm for 40 days are shown in Figures 10 and 11.

As a result, in the group exposed to 5 ppm, hardly any differences from the control group can be observed after 15 days except in the hematocrit values. However, after 30 days, it was observed that there were increases in the full blood specific gravity, the erythrocyte count, and the hematocrit value. In the group exposed to 1 ppm for three months, there were improvements in the results for the full blood specific gravity and the hematocrit value as the number of days of exposure increased. Although the hemoglobin count increased temporarily, it was observed at the end of the experiment to have returned to the same value it had before the experiment. In rats exposed to 0.5 ppm, during the first half of the exposure period there were no differences from the control group, but during the latter half of the period there was observed a tendency for the full blood specific gravity and the hematocrit value to increase.

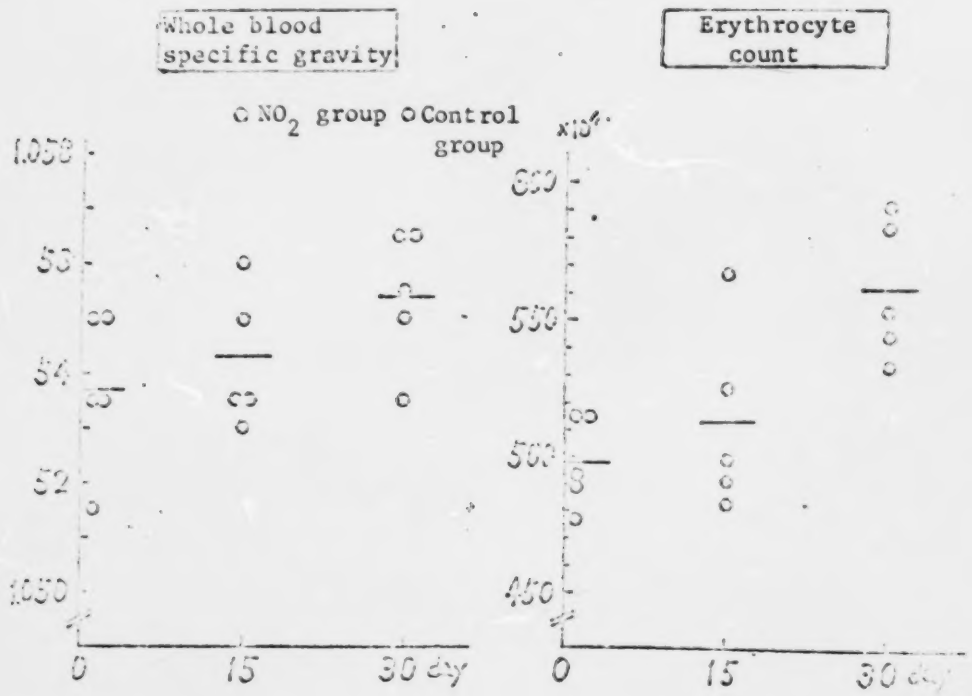

Figure 4. Blood findings in guinea pigs (1) (exposed to 5 ppm of NO_2)

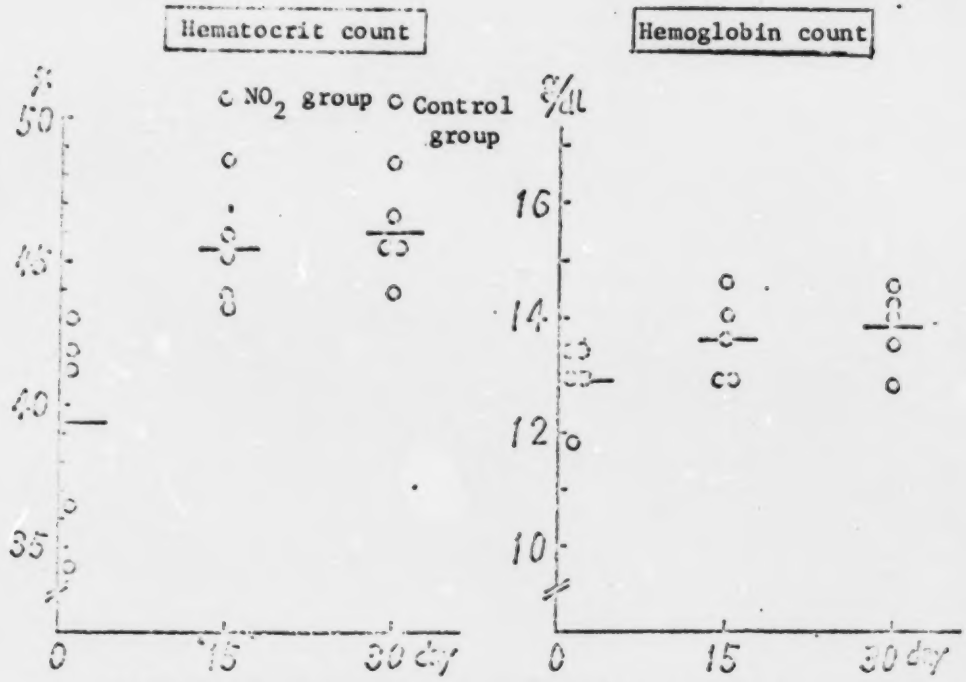

Figure 5. Blood findings in guinea pigs (2) (exposed to 5 ppm of NO_2)

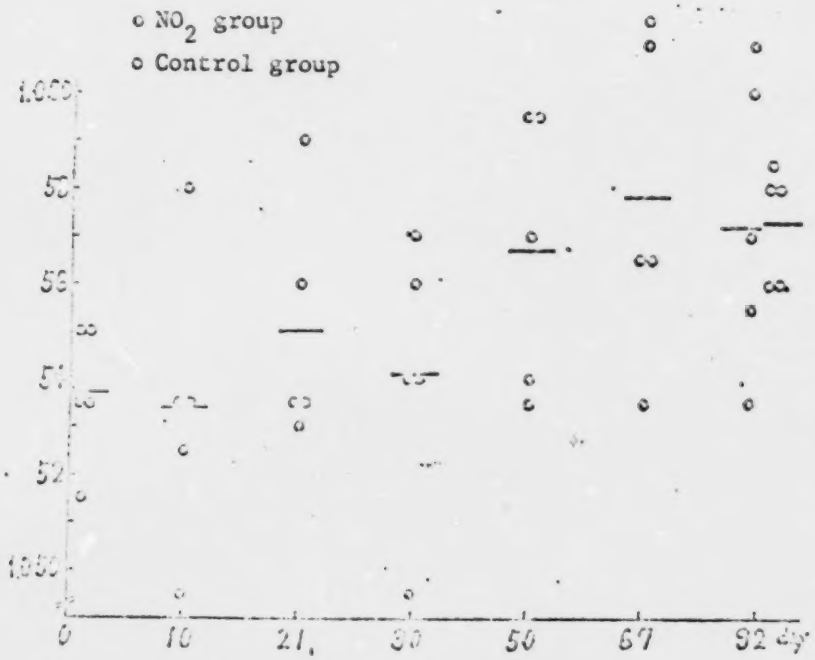

Figure 6. Blood findings of guinea pigs — Whole blood specific gravity (exposed to 1 ppm of NO_2)

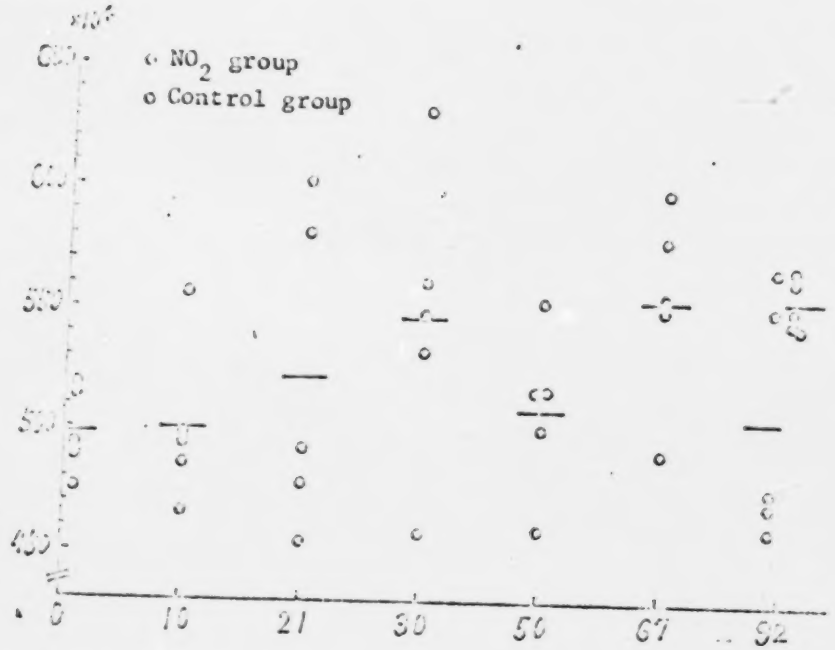

Figure 7. Blood findings in guinea Pigs — Erythrocyte count (exposed to 1 ppm of NO_2)

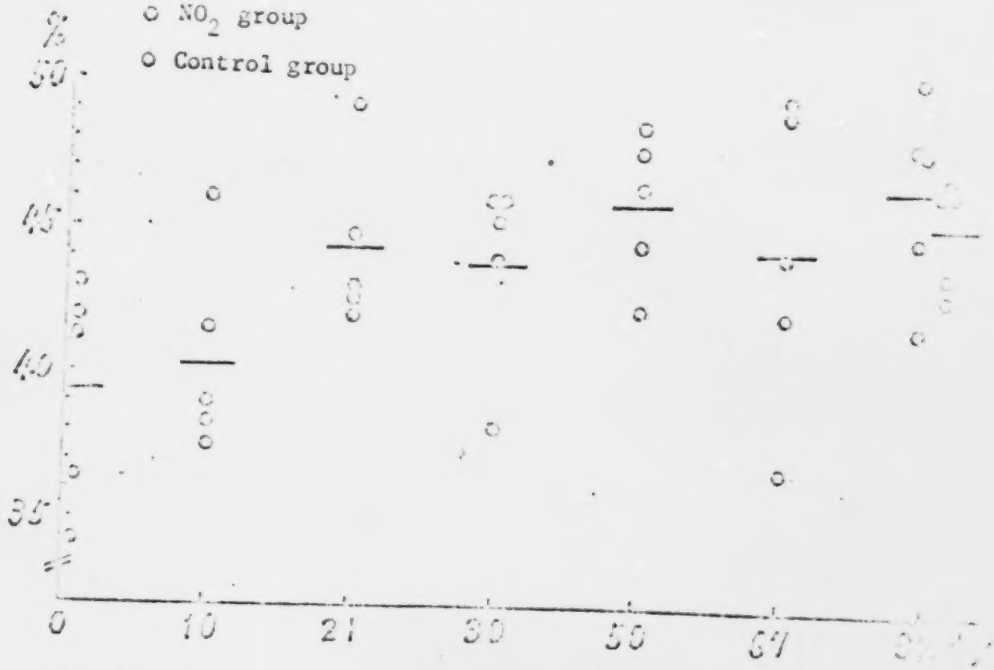

Figure 8. Blood findings in guinea pigs — Hematocrit count (exposed to 1 ppm of NO_2)

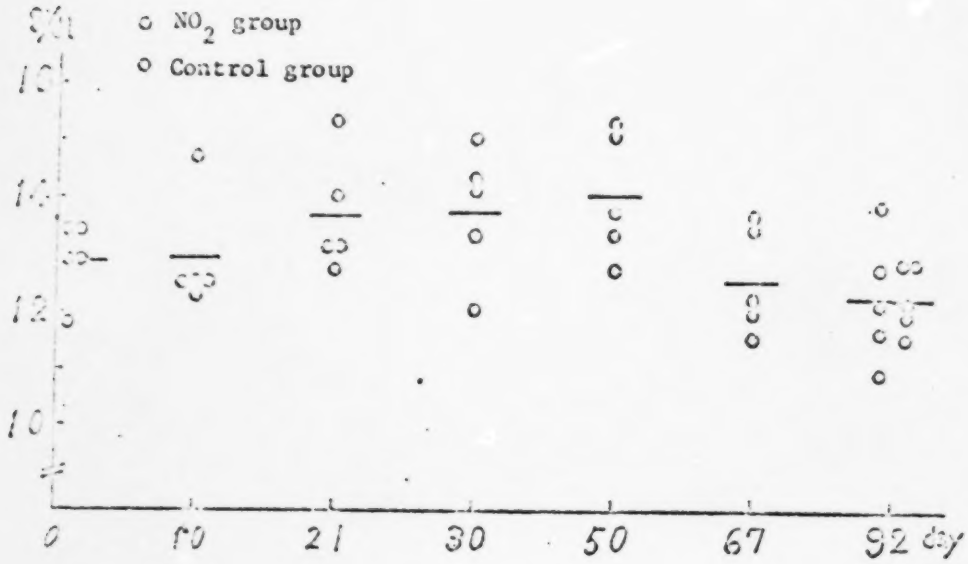

Figure 9. Blood findings in guinea pigs — Hemoglobin count (exposed to 1 ppm of NO_2)

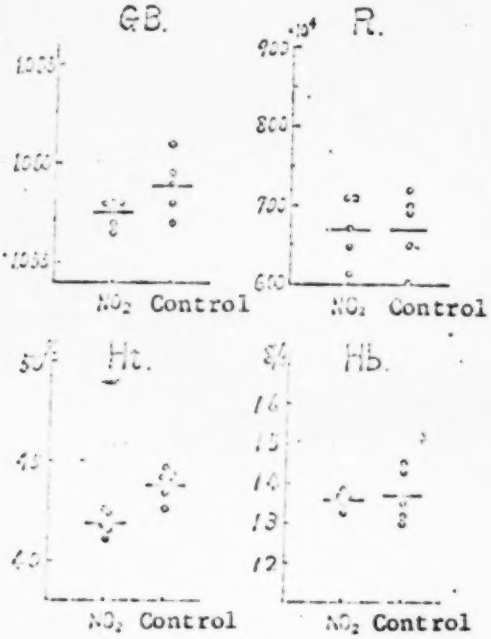

Figure 10. Blood findings in rats (18th day)

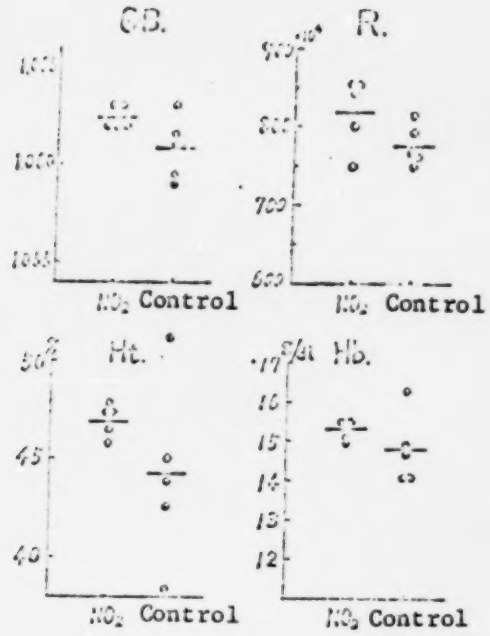

Figure 11. Blood findings in rats (39th day)

3. Influence on Respiratory Organs

3.1. Concerning the lung histamine contents

Studies were made concerning the phenomenon that histamine is liberated in the lung tissues as a result of irritation by nitrogen dioxide. In Figures 12 and 13 are shown the changes in the lung histamine contents together with the progress of exposure in the group exposed to 5 ppm for one month and in the group exposed to 1 ppm for three months. Studies were also made of the transfer into the blood of the hismatine liberated in the lung tissues. These results are given in Figure 14. The mean values for the above-mentioned results are shown in summary form in Figure 15.

It was observed as a result that the histamine contents in the lung tissues of the group exposed to 5 ppm for one month increased significantly on the 30th day. In the group exposed to 1 ppm for three months, there was

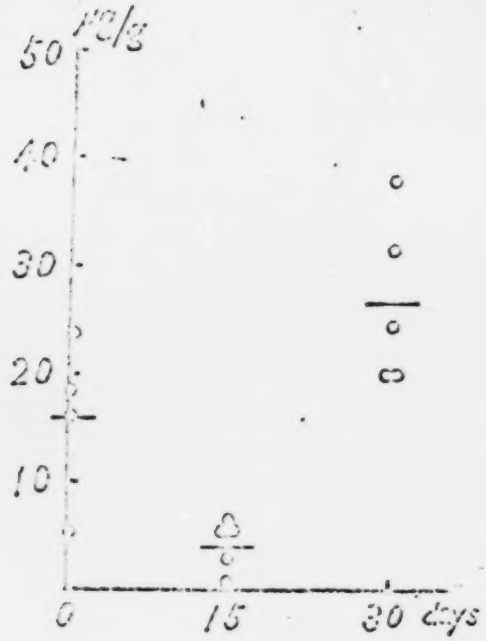

Figure 12. Lung histamine contents in group exposed to 5 ppm of NO_2

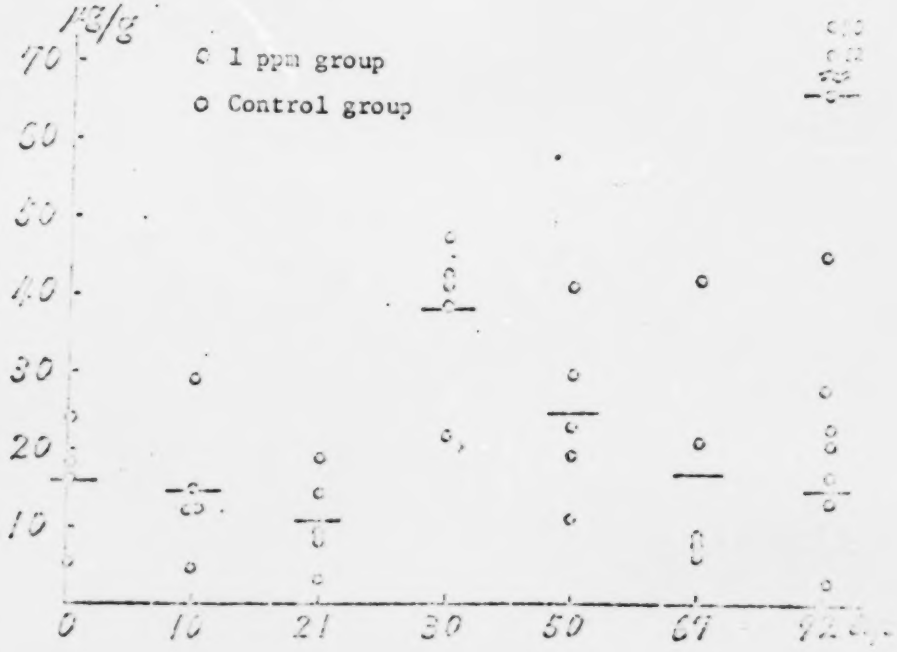

Figure 13. Lung histamine contents in group exposed to 1 ppm of NO_2

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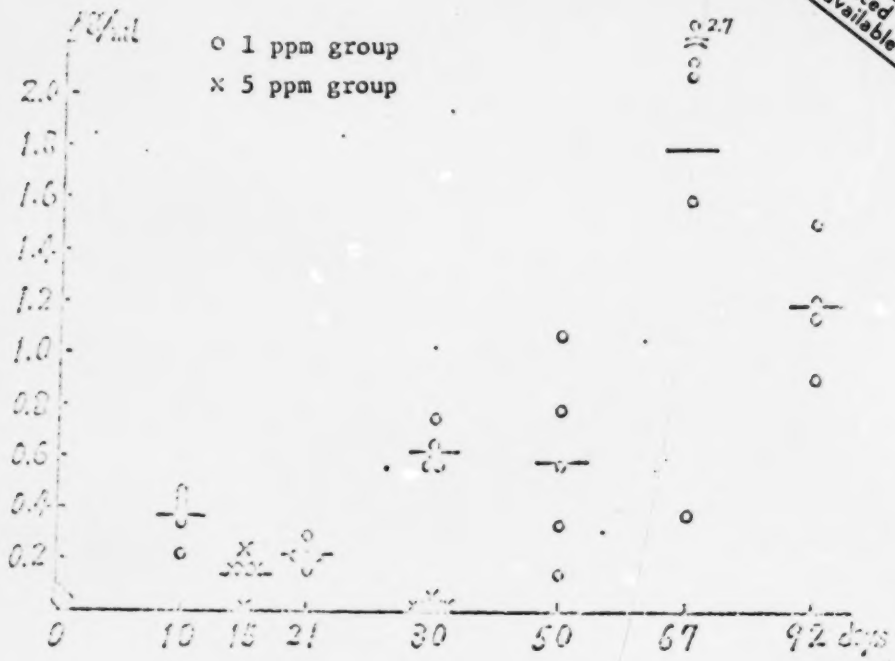

Figure 14. Blood histamine contents in groups exposed to NO_2

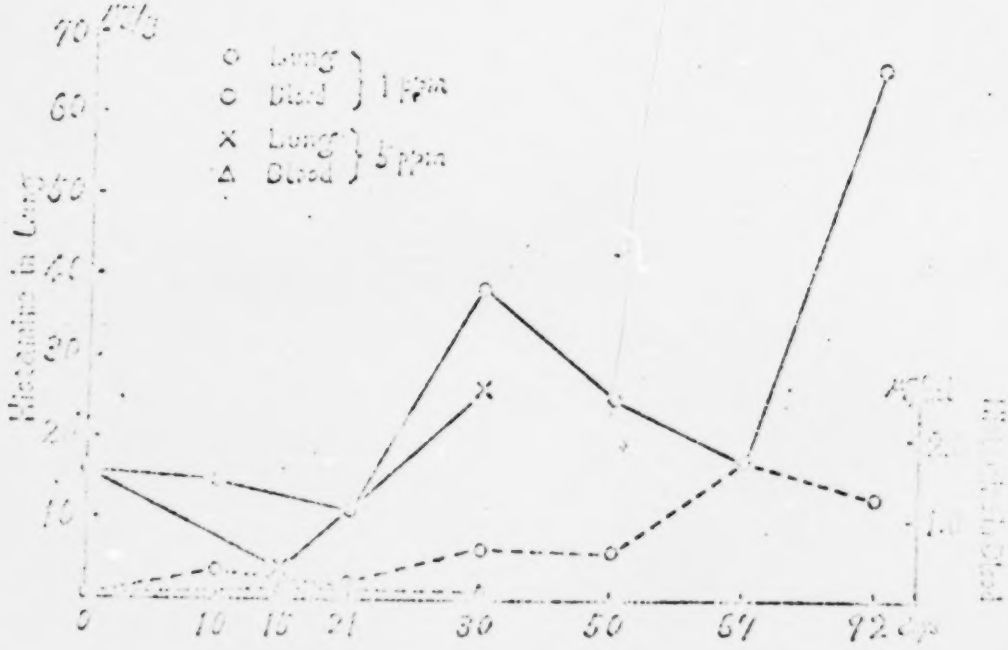

Figure 15. Shifts in lung and blood histamine contents after exposure to NO_2

Figure 16. Lung histamine contents in rats

in fact a lower value during the initial period. A temporary increase then occurred around the 30th day. Subsequently it was observed that a pronouncedly high value occurred after three months, although there were some fluctuations from time to time.

The histamine contents in the blood showed extremely low values in comparison with the contents in the lung tissues. In exposure to 5 ppm for one month, there were almost no changes at all, but in the group exposed to 1 ppm for three months there was a gradual increase with the progress of the exposure, and there was a tendency for the values to correspond to the shifts in the histamine in the lungs.

The histamine contents in rats are characteristically lower than those in guinea pigs. As is shown in Figure 16, it was not possible to find any effects of the nitrogen dioxide.

3.2. Concerning the lung weight and moisture contents

The occurrence of pulmonary edema as a result of irritating gases is often seen when extremely high concentrations of gases are inhaled. In connection with the phenomenon of increased amounts of free histamine in the lung tissues, mentioned in the previous section, there is interest in the hypertrophy of the lung tissues and the amounts of their moisture contents as a possible method of finding out whether the lungs are irritated by minute amounts of nitrogen dioxide.

In Figures 17, 18 and 19 are shown the wet weights of the lungs in terms of percentage of the total body weight for the group exposed to 5 ppm for one month, the group exposed to 1 ppm for three months, and the group exposed to 0.5 ppm for 40 days.

It is clear from these results that the lung weight is greatly influenced by inhalation of nitrogen dioxide and that there were pronounced increases in weight in both the group exposed to 5 ppm and the group exposed to 1 ppm.

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Figure 17. Lung weights of guinea pigs (percentage of total body weight) (group exposed to 5 ppm of NO_2)

However, it seemed that no particular effects could be seen in the group of rats exposed to 0.5 ppm.

The correlations between the lung weights and the histamine contents are shown graphically in Figures 20 and 21 for the groups exposed to 1 ppm and to 0.5 ppm. As the number of days of exposure increased, the group exposed to 1 ppm during the initial period displayed a strong tendency for the lung weight to increase in comparison with the control group, but it was observed that this gradually changed into a tendency for the histamine contents to increase.

Mice were exposed to 1 ppm for three months, and the moisture contents in the lungs were sought during this period. The results are shown in Table 5 and Figure 22. No differences from the control group could be observed.

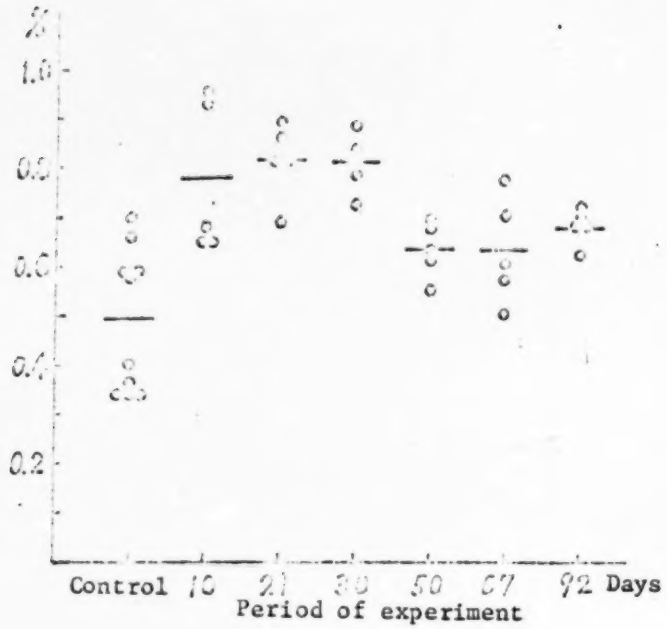

Figure 18. Lung weights of guinea pigs (percentage of total body weight) (group exposed to 1 ppm of NO_2)

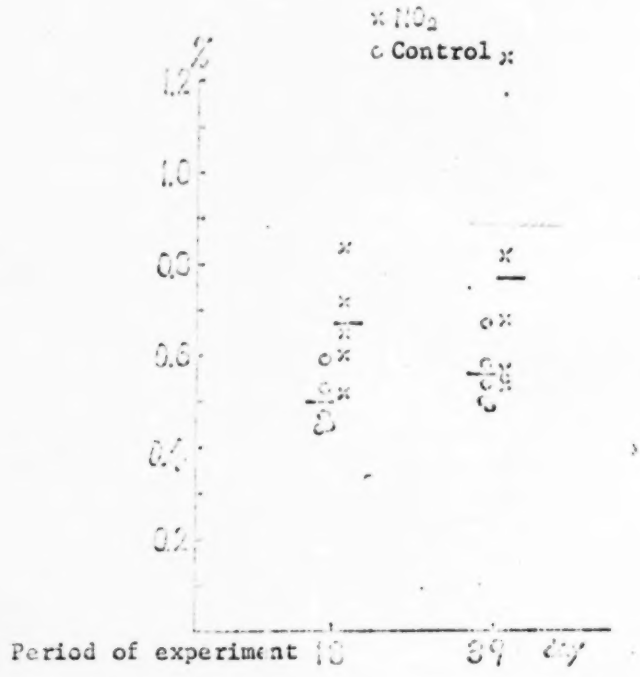

Figure 19. Lung weights of rats (percentage of total body weight)

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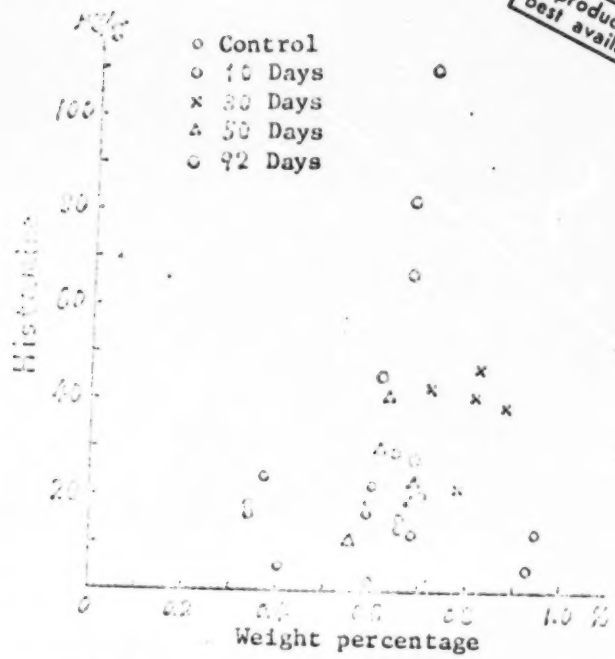

Figure 20. Relationship between lung weight and lung histamine contents (guinea pigs exposed to 1 ppm of NO_2)

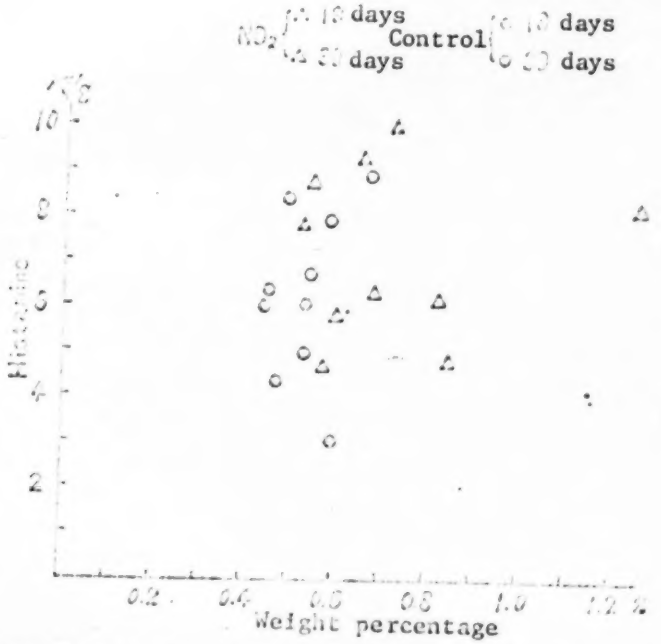

Figure 21. Relationship between lung weight and lung histamine contents (rats, exposed to 0.5 ppm of NO_2)

TABLE 2. MOISTURE CONTENTS IN LUNGS OF MICE (1 ppm OF NO_2)

(1 1/2 24hr)

	Control group	16th day	31st day	47th day	61st day	82nd day
Lung moisture contents (%)	78.54	75.40	78.06	78.34	77.16	78.61
	76.37	75.75	78.00	80.49	79.21	77.57
	79.66	78.93	77.35	80.18	78.70	78.47
	74.85	73.46	76.13	78.50	72.68	75.05
	78.82	77.78	76.20	74.56	79.37	76.71
						77.03
						77.11
Mean	78.04	76.66	77.96	78.37	77.46	77.33
	±1.32	±1.60	±0.84	±2.44	±2.70	±0.97

DISCUSSION AND SUMMARY

Environmental pollution caused by the nitrogen dioxide contained in automobile exhaust gases has caused problems in the state of California in the United States, and countermeasures against it appear to be under way. In Japan, we are still at the stage where countermeasures against automobile exhaust gases are just barely beginning to be taken. In the not-too-distant future, it is imagined that it will become necessary to take far more positive steps than are being taken at present against automobile exhaust gases as an important element in environmental pollution in the cities, on account of the growing congestion in urban areas and the growing amounts of automobile traffic.

Nitrogen dioxide is frequently mentioned in labor sanitation as an irritating gas which causes swelling of the alveoli of the lungs and pulmonary edema. The permissible concentration in working environments is regarded as being 5 ppm. There is much debate about whether this is a suitable concentration or not, and many have expressed the opinion that it ought to be suppressed to

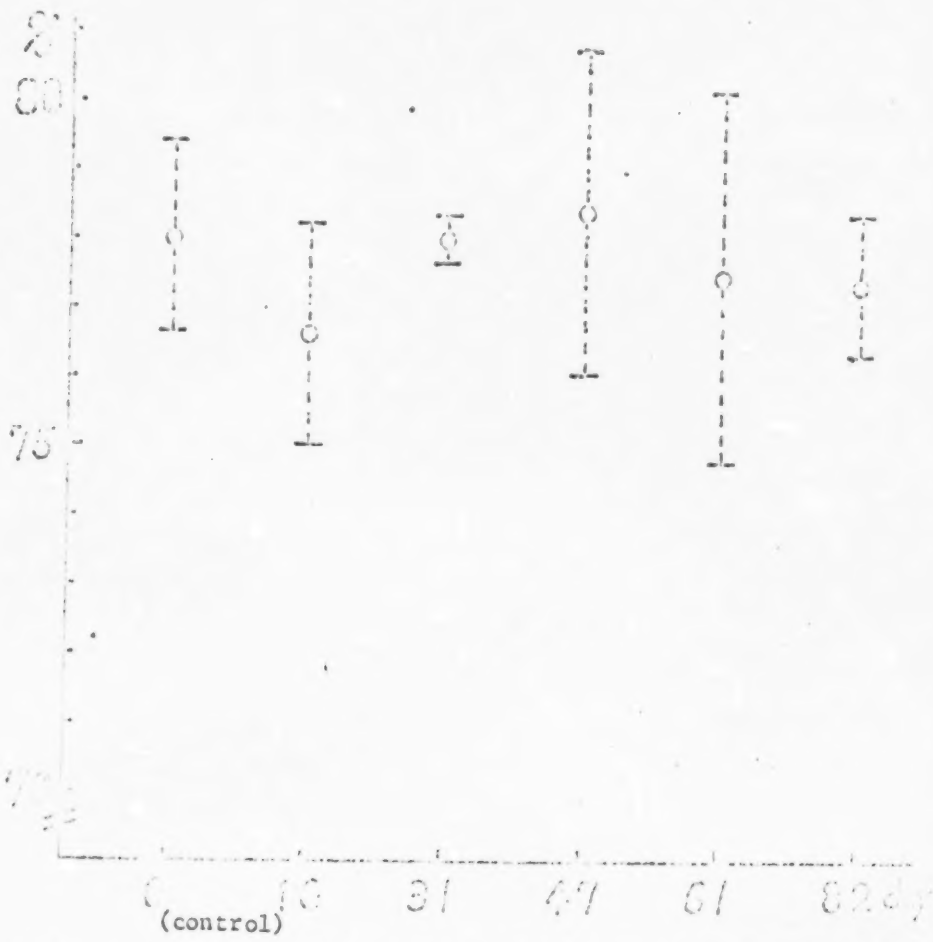

Figure 22. Moisture contents in lungs of mice (1 ppm of NO_2) (shows M + SD)

a lower level. As far as nitrogen dioxide pollution in the living environment in general is concerned, more careful attention certainly ought to be paid to it than to other pollutants.

Quite recently, there have been a few reports from Japan and overseas concerning the effects of minute amounts of nitrogen dioxide on living organisms. However, it is quite difficult to grasp significant differences in the vital reactions of organisms at the concentration levels which exist

in atmospheric pollution, and we have thus far been informed about only a small part of the effects. We also, for our part, have attempted a few studies concerning the effects of minute amounts of nitrogen dioxide on living organisms and have obtained the results outlined above. There were some inadequacies in our experimental methods, the periods of the experiments, and in the selection of the experimental animals, and follow-up studies will have to be made concerning some points. At any rate, let us here attempt some discussion of the results obtained.

First of all, concerning the influence on the growth, in studies concerning various concentrations, no differences from the control groups could be seen in the body weight increase. No doubt this result may be connected with the length of the periods of the experiments. At any rate, the effects of inhalation of nitrogen dioxide did not come to the point of appearing in the form of a comprehensive biological phenomenon such as inhibition of growth. However, when the durability of the physical strength of these animals was studied by the swimming method, it was observed that their physical strength declined proportionally to the length of the period of exposure. The manner in which loads are applied in the swimming method is one in which brings the entire body into action. There is also a large amount of respiration. Thus, a large amount of respiration. Thus, it is believed that the harmful properties of nitrogen dioxide towards the respiratory organs appeared vividly in the results.

As for the blood findings, there was generally observed an improvement of the blood properties regardless of the concentrations of nitrogen dioxide to which the animals were exposed. In this connection, one must naturally take into consideration the improvements in these findings accompanying the progress of growth of the experimental animals. However, the influence of nitrogen dioxide on the gas metabolism in the lungs cannot be ignored. In addition, the question of the hemotropic properties of nitrogen dioxide has recently been raised. Thus, it is possible to imagine that it may be connected also with inhibitions of the production of oxidized hemoglobin. Improvements in the blood properties such as those indicated by the experimental

results may be considered to be initial phenomena occurring as the result of the effects of this sort of toxic substances producing improvement in the metabolism. However, as far as this phenomenon is concerned, it would be somewhat problematical to come to a conclusion on the basis of these results along, and we believe that further discussion must await the results of prolonged experimental studies and of hematological studies.

Next, studies were made of the phenomenon of liberation of histamine in the lung tissues, of the lung weights, and of the lung moisture contents in order to discover the disturbances occurring in the respiratory system when it has been directly affected. Interesting phenomena were observed concerning these matters. Ordinarily, histamine is believed to be in an inert state in the tissues linked with protein, while some of it is active in the blood plasma, where it is free in the form of ions. It is naturally to be expected that there will be an increase in the amount of free histamine in the tissues upon chemical attack by irritating gases such as nitrogen dioxide. This can be considered in connection with the acceleration of inflammation, such as the phenomena of swelling of the alveoli of the lungs and the occurrence of pulmonary edema. It is further supposed that there will occur increased amounts of liquid exuded in the lung tissues as well as hypertrophy of the lung parenchyma.

The experimental results also show pronounced increases in the histamine contents in the lungs and that the amount of free histamine in the blood also increases gradually as the period of exposure is prolonged. Thus, it is indicated that nitrogen dioxide has pronounced irritating effects on the respiratory organs. As was mentioned in the results concerning the changes in the moisture contents in the lungs of mice, it was not always possible to discover effects of nitrogen dioxide. However, in the studies of the lung weights of guinea pigs, pronounced increases in weight were observed as a result of exposure. Along with the dynamics of free histamine in the lung tissues, this also was believed to be an interesting phenomenon in studying the influences of chronic exposure to nitrogen dioxide on living organisms.

Thus, as a result of the several types of experimental studies which we attempted, it was found that, although there were some differences in the manner in which the effects manifested themselves depending upon the conditions of exposure to low-concentration nitrogen dioxide, the periods of exposure, and the kinds of animals used, some of the results obtained served to reinforce our concepts, particularly concerning the intensity of the irritation to the respiratory organs. Even though it would be problematical to apply these results obtained with small animals directly in a discussion of the effects on the human organism, it is believed that the results will nevertheless be of interest when considering countermeasures in the future.

In studies of impairments of the respiratory organs caused by atmospheric pollution in the past, the main emphasis has been placed on the effects of sulfurous acid gas. However, if urban environmental pollution continues to be aggravated further by automobile exhaust gases, as was mentioned above, it will naturally be necessary to focus our attention also on nitrogen dioxide, which is one of their chief components. The authors will be happy if this report will be of assistance in formulating future countermeasures.